US005990178A

United States Patent [19]

Ninkov

[11] Patent Number: 5,990,178

[45] Date of Patent: Nov. 23, 1999

[54] **PHARMACEUTICAL COMPOSITIONS SUITABLE FOR USE AGAINST HISTOMONIASIS**

[75] Inventor: **Dusan Ninkov**, Amsterdam, Netherlands

[73] Assignee: **Ropapharm B.V.**, Zaandam, Netherlands

[21] Appl. No.: **09/162,161**

[22] Filed: **Sep. 29, 1998**

[30] **Foreign Application Priority Data**

Sep. 30, 1997 [EP] European Pat. Off. 97203004

[51] **Int. Cl.**[6] **A61K 31/05**; A61K 35/78

[52] **U.S. Cl.** **514/731**; 424/195.1

[58] **Field of Search** 514/731; 424/195.1

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

53-066420 6/1978 Japan .

WO 97/01348 1/1997 WIPO .

OTHER PUBLICATIONS

Abstract 1923, "Carvacrol", The Merck Index and Encyclopedia of Chemicals, Drugs, and Bilogicals, Twelfth Edition, p. 308, 1996.

Abstract 9540, "Thymol", The Merck Index and Encyclopedia of Chemicals, Drugs, and Bilogicals, Twelfth Edition, p. 1604, 1996.

Primary Examiner—Phyllis G. Spivack

Attorney, Agent, or Firm—Young & Thompson

[57] **ABSTRACT**

The invention relates to pharmaceutical compositions comprising carvacrol and/or thymol as active agents in the veterinary field. The compositions according to the invention can be used in the treatment of histomoniasis, an infectious disease of poultry, mainly of turkeys, and in treatment against hemoflagellates.

6 Claims, No Drawings

PHARMACEUTICAL COMPOSITIONS SUITABLE FOR USE AGAINST HISTOMONIASIS

DESCRIPTION

1. Field of Invention

The invention relates to pharmaceutical compositions comprising carvacrol and/or thymol as active agents, a process for the preparation of such pharmaceutical compositions as well as their use in the human and veterinary field, e.g. against histomoniasis.

More in particular histomoniasis is an infectious disease of poultry, mainly turkeys, due to *Histomonas meleagridis* with intestinal and hepatic lesions and dark discoloration of the comb ("black-head"). In detail the trophozoites of *Histomonas meleagridis* and resulting lesions are confined to the cecum and liver. The infected cecum is enlarged and the mucosa becomes necrotic consisting of leather-like cheesy material. The parasites lie singly or in small groups in the spaces between the cells. From the mucosa, they can spread to the submucosa and muscle layers and eventually be carried to the liver via the portal blood. The liver has circular areas or necrotic tissue usually resulting in impaired function. Early liver lesions are small in size, spherical and cream-colored while older lesions are large with depressed dark centers and a pale periphery. Clinical signs include drowsiness, weakness, and sulfur-colored droppings. Transmission can be by ingestion of trophozoites or ingestion of *Heterakis gallinae* (nematode) egg containing the trophozoite. In the latter case the flagellated form of the *H.meleagridis* is ingested by the co-habitating *H.gallinarum* nematode. The Histomonas passes through the gut wall of the female worm and penetrates into the ovary. It multiplies in the ovary and invades the oocysts. When embryonated Heterakis eggs are ingested by the susceptible host, the Histomonas escapes into the lumen of the cecum. Young birds usually have an acute form of the histomoniasis disease while older birds may appear sick for several days prior to becoming emaciated. Heaviest losses occur at 3 to 12 weeks of age. Many other species of birds including quail and pea fowl are also susceptible to above infection. A treatment is possible with nitroimidazole as well as the following medicines Dimetriazol and Ronidazol. Separation of species and ages is vital in preventing this disease.

Further, the invention relates to pharmaceutical compositions, suitable against hemoflagellates. These parasites live in the blood, lymph, and tissue spaces and are typically transmitted from one host to another by blood-feeding arthropods. The most important genera are Trypanosoma and Leishmania. Infection in mammalian hosts occurs either through the bite of the infected arthropod (salivarian) or through contamination of the host's mucus membranes or abraded skin by the arthropod's infected feces (stercorarian).

The pharmaceutical compositions according to the invention are also suitable for combating inflammation diseases like pneumonia, nephritis, metritis, artritis, othitis, pharingitis, gastro-enteritis, sepsis caused by Salmonella spp, Pasteurella spp, *E.coli, Vibrio coli* etc. and any other inflammation in the organism of human and/or animals caused by the bacteria species, causing above-mentioned pathological diseases.

2. Problem Related to the Prior Art

The above-defined diseases are well known in the art, together with the relevant medications therefore. However, the prior art medications have been either forbidden on account of for example the presence of bioresidues in the meat of e.g. turkeys, its cancerogenic properties or they have become less active against the harmful microorganisms in question. Especially in the last decade many pathogenic microorganisms like *Salmonella typhimurium* DT 104 have build up considerable resistance to the marketed antibiotic products.

3. Description of the solution of the above-described problem

The primary component(s) to be applied in the compositions according to the invention is thymol (2-hydroxy-1-isopropyl-4-methylbenzene) and/or carvacrol (2-hydroxy-4-isopropyl-1-methylbenzene). Although above active compounds may have a synthetic origin, preferably the active compounds are applied in the form of an oil extracted from any of the plants, selected from the group consisting of *Origanum vulgaris, Thymus vulgaris, Mentha piperita, Thymus serpilum, Saturea hortensis, Saturea montana, Saturea subricata, Carum corticum, Thymus zugus, Ocimum gratisimum, Moranda pungtata, Mosla japanoica* and *Salvia officinalis.*

The pharmaceutical compositions according to the invention may comprise a pharmaceutically acceptable carrier, preferably of natural origin. Representatives of such carriers are generally known in the human and veterinary pharmaceutical field. Examples of such carriers are lactose, honey, laurel, vaselin, paraffin, starch products, calcium carbonate, etc.

The pharmaceutical compositions may have any form suitable for its application, for instance the form of a water-soluble solution and a powder in the case of the treatment of histomoniasis and in the form of an injectable solution in the case of the above-defined inflammations.

The content of active agent in the pharmaceutical compositions according to the invention, which in fact does also depend on its pharmaceutical use, may vary between wide limits. Preferably the active agent in the form of thymol and/or carvacrol is present in an amount of 1–10% by weight, most preferably 2–5% by weight, calculated on the total weight of the pharmaceutical composition.

Further, to the active agent according to the invention also other active substances, preferably of natural origin, can be used. Such substances may have bacteriological, fungicidal, adstrigidic etc. properties.

The way of application of the pharmaceutical compositions according to the invention depends on their form. For instance, the treatment of histomoniasis may be carried out by means of a water soluble solution or powder per oral, whereas the treatment of the above-defined inflammation diseases may be carried out by means of an injectable solution in an intramuscular, subcutaneous, intraperitoneal and/or intravenous way.

Examples of forms of pharmaceutical compositions according to the invention are for instance:

1) Powder form

The following composition in powder form may be used in the treatment of poultry, e.g. turkeys, against histomoniasis.

$CaCO_3$: 20–25 wt. %

Magnesium stearate: 3–5 wt. %

Potato starch: 25–30 wt. %

Dextrose: 45–50 wt. %

Caivacrol and/or Thymol*: 3–4 wt. %

* In this case carvacrol and/or thymol are applied as an etheric oil extracted from the above-mentioned plants: 6–7 wt. %

2) Water soluble solution

The following composition may be used in the treatment of poultry against histomoniasis.

Double distilled water: 4–5 wt. %

Polysorbate: 60–65 wt. %

Monoethylene glycol: 3–35 wt. %

Caivacrol and/or Thymol**: 3–4 wt. %

** In this case carvacrol and/or thymol are applied as an etheric oil extracted from the above-mentioned plants: 6–7 wt. %

3) Injectable solution

The following composition may be used in the treatment of inflammatory diseases like pneumonia etc.

Double distilled water: 40–55 wt. %

Emulgator 686: 2–3 wt. %

Polysorbate: 40–43 wt. %

Carvacrol and/or Thymol***: 1–3 wt. %

*** In this case carvacrol and/or thymol are applied as an etheric oil extracted from the above-mentioned plants: 3–5 wt. %

The following example is merely given as an illustration of the invention and should not be interpreted in a restrictive way.

Example 1

The following powder composition was applied:

$CaCO_3$: 25 wt. %

Magnesium stearate: 5 wt. %

Potato starch: 25 wt. %

Dextrose: 41 wt. %

Carvacrol and/or Thymol: 4 wt. %

In a farm 90 turkeys, suffering from both histomoniasis and rhinitis were treated with the above-defined composition in a dosis of 5 g/kg over 10 days. At the first day of the treatment only one turkey died. The others recreated quickly, and only some of them still had rhinitis.

For comparison purposes 90 turkeys of the same group as above, also suffering from both histomoniasis and rhinitis, were treated by the marketed product "Bayril". However, 20 turkeys died without showing sulfur-coloured feces before. At necropsy of four of these turkeys, they showed the typical picture of typhlohepatitis in different degrees of severity.

I claim:

1. A method of treating a malady which is diseases induced by hemoflagellates in poultry, comprising administering to poultry suffering therefrom, an effective amount of at least one member selected from the consisting of thymol and carvacrol, said amount being effective to treat said malady.

2. A method as claimed in claim 1, wherein said member is administered in a composition containing 1 to 10% by weight of said member.

3. A method as claimed in claim 1, wherein said member is administered in a composition containing 2 to 5% by weight of said member.

4. A method of preventing a malady which is diseases induced by hemoflagellates in poultry, comprising administering to poultry an effective amount of a member selected from the consisting of thymol and carvacrol, said amount being effective to prevent said malady.

5. A method as claimed in claim 4, wherein said member is administered in a composition containing 1 to 10% by weight of said member.

6. A method as claimed in claim 4, wherein said member is administered in a composition containing 2 to 5% by weight of said member.

* * * * *

US006106838A

United States Patent [19]

Nitsas

[11] Patent Number: 6,106,838

[45] Date of Patent: Aug. 22, 2000

[54] **PHARMACEUTICAL COMPOSITIONS CONTAINING HERBAL-BASED ACTIVE INGREDIENTS; METHODS FOR PREPARING SAME AND USES OF SAME FOR MEDICAL AND VETERINARY PURPOSES**

[76] Inventor: **Fotios A. Nitsas**, Kristoni, GR-611 00 Kilkis, Greece

[21] Appl. No.: **08/981,946**

[22] PCT Filed: **Jun. 27, 1996**

[86] PCT No.: **PCT/GR96/00016**

§ 371 Date: **Dec. 29, 1997**

§ 102(e) Date: **Dec. 29, 1997**

[87] PCT Pub. No.: **WO97/01348**

PCT Pub. Date: **Jan. 16, 1997**

[30] **Foreign Application Priority Data**

Jun. 29, 1995 [GR] Greece 950100249

[51] **Int. Cl.**[7] **A61K 35/78**; A01N 25/00; A23L 1/222

[52] **U.S. Cl.** **424/195.1**; 424/404; 424/405; 424/439; 426/2; 426/53; 426/489; 426/542; 426/651; 426/655; 514/885

[58] **Field of Search** 424/195.1, 439, 424/404, 405; 426/489, 651, 542, 2, 655, 53; 514/885

[56] **References Cited**

PUBLICATIONS

Baser et al. J. Essent. Oil Res. vol. 5 (6), pp. 616–623 (abstract enclosed), 1993.

Lagouri et al. Zeitschrift fuer Leensmittel Unter. und Fors. vol. 197 (1), pp. 20–23, 1993.

Akguel et al. Nahrung. vol. 32 (2), pp. 201–203, 1988.

Sarer et al. Planta Med. vol. 46 (4), pp. 236–239 (abstract enclosed), 1982.

Van den Broucke et al. Planta Med. vol. 38 (3), pp. 264–266 (abstract enclosed), 1980.

Assaf et al. Planta Med. vol. 53 (4), pp. 343–345 (abstract enclosed), 1987.

Primary Examiner—Leon B. Lankford, Jr.
Assistant Examiner—Christopher R. Tate
Attorney, Agent, or Firm—St. Onge Steward Johnston & Reens LLC

[57] **ABSTRACT**

Methods for treating and preventing coccidiosis in poultry, and inflammation, infection, and diarrhea in mammals are provided utilizing an antimicrobial pharmaceutical composition comprising an herbal essential oil which contains thymol and carvacrol as its main ingredients. The essential oil is preferably obtained from the genus Origanum, especially *Origanum vulgare* ssp. *hirtum*.

12 Claims, No Drawings

PHARMACEUTICAL COMPOSITIONS CONTAINING HERBAL-BASED ACTIVE INGREDIENTS; METHODS FOR PREPARING SAME AND USES OF SAME FOR MEDICAL AND VETERINARY PURPOSES

FIELD OF THE INVENTION

The present invention relates to the preparation of various forms of pharmaceuticals for medical and veterinary uses, said pharmaceuticals comprising as active ingredients special herbal essences, capable of substituting antibiotics and sulphamide based drugs, due to their important activity against germs causing inflammations, infections and diarrhoea in humans and animals.

DESCRIPTION OF RELATED ART

The presently used methods for treatment of inflammations, infections and diarrhoea in humans and animals rely on the use of pharmaceuticals that contain antibiotics and sulphamides. It is known that these pharmaceuticals are often the cause of severe short and long term side effects, for example the accumulation of bioresidues. In addition, microorganisms that are to be combatted by these pharmaceuticals develop with time a resistance to these drugs, thus reducing the efficiency of the treatment.

This is because the living organism of humans or animals is incapable of fully assimilating or rejecting these chemicals, resulting in accumulation of chemical in the organism and causing serious side effects exemplified by hereditary changes or sensitivity to microorganisms against which these chemicals had been used.

It is to be noted that animals are more subjected to frequent use of antibiotics than humans. It is known that antibiotics against infections and inflammations are regularly administered on a daily basis to livestock by enrichment of livestock foodstuffs (poultry dough) to prevent diarrhoea and coccidiosis.

This is because in their effort to preserve their livestock capital from infectious, inflammatory and diarroeic diseases, stock-farmers inconsiderately resort to frequent use of these pharmaceuticals and this practice, although helpful in the treatment of said diseases, results in consumer products being charged with elevated quantities of antibiotics.

The situation in humans is to a lesser extent but equally alarming, as many deaths are reported that are caused by prolonged treatment with antibiotics.

BACKGROUND OF THE INVENTION

The present invention refers to compositions containing essential oils that are as effective against inflammations, infections and diarrhoea as antibiotics and sulphamides, but differ essentially from the latter (and this is due to the herbal origin of these essential oils) in that they are fully assimilable or rejectable by the living organism, thus avoiding bioresidues and side effects.

These substances originating from herbal essences, in admixture with inert substances according to methods that are described below, constitute the essential ingredients of pharmaceutical compositions for medical and veterinary use, said compositions having properties of full and effective prevention of and treatment of various inflammations, infections and diarrhoea, while being safe for the environment and the organism (human or animal).

The substances that constitute the essential ingredient of said pharmaceutical compositions which are one of the

objects of the present inventions are herbal essences with high contents in thymol, carvacrol and tannin.

These substances can all be obtained from herbs like *Thymus vulgaris, thymus serpilum, saturea hortensis, saturea montana, saturea subricata, carum coptimum*(India), *thymus zygus* (Spain), *ocimum gratisum* (Southern France and Africa), *moranda puntata* (North America), *mosia japanoica maxinowisz* (Japan), *salvia officiaiatis* and the like.

Herbs of the Labiatae family, which have been widely used as spices for flavouring dishes and beverages, are known to contain high amounts of thymol and carvacrol. The most common among them, thyme and oregano, differ from each other mainly in the content ratio of thymol and carvacrol. The essential oil obtained from thyme, which is known to possess antifungal and antimicrobial activity, has a higher content in thymol; on the contrary, herbs of the genus origanum contain predominantly carvacrol.

It was found that thymol alone as well as essential oils containing thymol in amounts corresponding to a carvacrol:thymol ratio of lower than 5:1, which show some antimicrobial activity on certain microorganisms, in particular *Rhizobium legumirosarum*, have the disadvantages that they have mediocre activities on strains of *Staphylococcus aureus* and an inferior activity on *Bacillus subtilis*.

Further, isolated thymol showed a good antimicrobial activity on *Escherichia coli*, while essential oils and their mixtures containing relatively low levels of carvacrol (having a carvacrol:thymol ratio lower than 5:1) resulted in a significantly reduced activity on the same microorganism, leading to the assumption that an antagonistic effect between the other ingredients of the essential oils may affect their activity on *E. coli*.

On the contrary, carvacrol alone, which had a significant antimicrobial activity on *Staphylococcus aureus*, had an inferior activity on *Escherichia coli, Bacillus subtilis* and *Salmonella typi*.

There is thus a continuing need of herbal based pharmaceutical compositions which overcome the above mentioned drawbacks.

SUMMARY OF THE INVENTION

The herbal essential oil contained in the pharmaceutical composition of the present invention is characterised in that the total amount of thymol and carvacrol comprised therein is at least 55%, preferably 70% by weight and in that the ratio of carvacrol to thymol is at least 10:1.

The above given ratio of carvacrol to thymol is found to provide surprising antimicrobial properties, when compared to the corresponding properties of essential oils having a ratio lower than 10:1 and of isolated thymol and carvacrol.

A high total content of thymol and carvacrol in the essential oils is of great significance. When they are contained in the herbs in high amounts, they can be obtained from the leaves and flowers of the herbs by means of simple processes in higher yields. It is therefore possible and preferable to obtain the essential ingredients by a process as simple as steam distillation, which reduces costs at a considerable extent.

Moreover, a high concentration in the essential ingredients has the economical advantage that the essential oil may be used in small quantities to obtain the desirable effects.

Well-balanced overall results were obtained by essential oils having a ratio of carvacrol to thymol in a range of 30:1 to 150:1. The best antimicrobial activities have been observed in the range of 40:1 to 110:1.

A further advantage in the use of the essential oils according to the invention in comparison to the use of isolated carvacrol is the fact that the former are obtainable by simple processes, whereas the isolation of pure carvacrol requires at least an additional chromatographic separation.

Antimicrobial tests were performed on standard microorganisms including *E.coli, Staphylococcus aureus, Salmonella typhi, Rhizobium leguminosarum, Bacillus subtilis* and of the Eimeria group.

The high levels of thymol and carvacrol required by the present invention are obtainable by steam distillation of carvacrol-rich herbs belonging preferably to the genus origanum. The essential oils obtained have a total amount of thymol and carvacrol comprised therein is at least 55%; most of the specimen examined had a total amount in a range between 70 and 88% by weight and a carvacrol to thymol ratio of 40:1 to 110:1 (see Table 1).

Origanum vulgare ssp. *hirtum* (a wild growing subspecies of *Origanum vulgare*, growing in the Greek mainland) and *Origanum heracleoticum* (which grows on the island of Crete) are preferred because they contain thymol and carvacrol in levels of higher than 55%, that is much higher than the herbs listed in the introduction above can provide.

The origin of the herbs used may account for the differences in the compositions of the essential oils obtained. Referring to Table 1, it may be generally concluded that the carvacrol predominance is more recognisable in herbs growing in Crete, and that the higher absolute thymol levels are measured in essential oils originating from the island of Euboea. The differences in the compositions may depend on the climatic conditions and their variations.

The essencial oils necessary for the performance of the present invention are preferably obtained from origanum hyrtum and origanum heracleoticum by steam distillation. To do so, the leaves and flowers of the herb, after being dessicated, are firstly charged in an extractor equipped with two tubes for steam and oil passage respectively.

The water vessel positioned below the dessicated herbal leaves and flowers is heated at 100° C. under pressure of 20 kp/cm^2. The water steam, upon contacting with the dessicated herbs, extracts the essence which is collected and flows from the bottom of the extractor towards a product vessel. This process takes about 3 hours, yielding 5–6 kg of herbal oil (essence) per 100 kg of dessicated herbs.

Following this first distillation step, the herbs are reextracted in the same extractor, replacing the water by a water-alcohol mixture (20% water-80% alcohol), which is again heated at 100° C. This is the second distillation step.

The liquid collected in the product vessel from the bottom of the extractor at the end of the second distillation step is the starting material from which tannin is obtained as follows: The content of the vessel is heated at 80° C. to evaporate the alcohol. After about one hour, the alcohol has fully evaporated and the residue comprises tannin.

The product obtained from the above described steam distillation was subjected to gas chromatography-mass spectroscopy and the analytical results are shown in Table 1.

A commercially available origanum oil was analysed as well, for comparison. It is noted that the origanum oil, which is obtained by cultivated oregano herbs, contains very low levels of carvacrol, being untypical for origanum, but high levels of its precursor γ-terpinene (40 wt %) and significant amounts of camphene (5.4 wt %), limonene (4.1 wt %), α-pinene and α-terpineol.

TABLE 1

Specimen	Essential oil	Carvacrol (C)	Thymol (T)	Total (C + T)	Ratio (C/T)
1	*Origanum vulgare* ssp. *hirtum*	83.3	1.1	84.4	75.7
2	*Origanum hyrtum*	79.6	2.5	82.1	31.8
3	*Origanum hyrtum*	72.0	1.2	73.2	60.0
4	*Origanum heracleoticum*	85.3	0.9	86.2	94.8
5	*Origanum heracleoticum*	84.4	0.6	85.0	140.7
6	*Origanum heracleoticum*	82.2	0.8	83.0	102.8
Comparison	*Origanum oil* (commerical)	0.4	31.8	32.2	<1

Measurements of antimicrobial activities were carried out using the essential oils as obtained by steam distillation, as well as mixtures of said oils and mixtures of *Origanum vulgare* ssp. *hirtum* oil with *Origanumdictamnus oil* (wild growing in Crete) which has a carvacrol and thymol content of 62.4% and 0.4% respectively (carvacrol to thymol ratio= 152). As mentioned above, good activities were measured at carvacrol:thymol ratios of 30:1 to 150:1 and the best results are obtained in the respective ratio within the range of 40:1 to 110:1 by weight.

It is noted that since carvacrol and thymol are isomeric compounds, their weight ratio corresponds to their molar ratio.

Though it was established that neither γ-terpinene nor p-cymene, the precursors of carvacrol and thymol (which are comprised in the essential oils comprised in the pharmaceutical composition of the invention in levels of 2 to 11% by weight) had any antimicrobial activity, their presence may contribute to the synergistic effect observed with the pharmaceutical composition according to the invention.

According to one aspect of the present invention, there is provided a substitute for antibiotics to be regularly administered on a daily basis to poultry, in order to prevent diarrhoea and coccidiosis.

In another aspect of the present invention, the pharmaceutical composition can be successfully used for the prevention and treatment of coccidiosis, a contagious infection of poultry affecting the intestinal epithelium and causing enteritis and diarrhoea.

In a further embodiment these pharmaceutical compositions are used for the treatment of the following:

a. chronic mastitis or mastitis caused by Staphylococcus or Streptococcus,

b. dermal fungal infections and inflammations including infections and inflammations in vagina and/or uterus (humans and animals),

c. aural infections,

d. ophthalmic inflammations,

e. inflammations of lungs (pneumonia), and

f. inflammations of kidneys (nephritis).

The bactericidal and bacteriostatic activity of the essential oils of the invention was also examined in high dilutions. High bactericidal activities (on *Staphylococcus aureus*) at dilutions of 1:4000 were established, while the bacterial growth rate of the same microorganism was considerably decreased at dilutions of 1:10000 and even 1:50000.

Accordingly, in a preferred embodiment of the present invention, the pharmaceutical composition is used for the purification of water. In a concentration of about 10 to 20 ml

of a 5% aqueous solution of the essential oil per cubic meter water, the ingredients of the essential oils according to the present invention provide a bacteriostatic effect without impairing the odour and taste of drinking water. They can therefore be applied as a substitute for chlorine, which is still widely used by municipal water suppliers at levels which are not only questionable with respect to corrosion of the water supplying pipeline but also impart a disagreeable odour to drinking water.

In a further preferred embodiment the pharmaceutical composition of the invention comprises, in addition to the essential oils obtainable by steam distillation, the aqueous alcoholic extract of herbs belonging to the genus Origanum. This extract, which essentially consists of tannin and is obtained by the extraction of the distillation residues as described above, is preferably gained from *Origanum vulgare* ssp. *hirtum* and/or *Origanum heracleoticum.*

The resulting pharmaceutical composition comprising a mixture of the essential oils with the tannin is used in the treatment of diseases caused by pathogenic micro-organisms of the abdominal region. The diseases which may be treated by the pharmaceutical composition of this embodiment are caused by micro-organisms selected from salmonella, staphylococcus, pasteurella and *Escherichia cola.*

The following non limiting examples illustrate the invention. The percentages given represent weight percentages.

EXAMPLES 1 to 9

Examples 1 to 9 concern the preparation of pharmaceutical compositions comprising the essential oil of *Origanum vulgare* ssp. *hirtum* referred to above as Specimen 1.

Example 1

There is provided a pharmaceutical composition for medical and veterinary uses for the treatment of Salmonellasis, Staphylococciasis, Pasteuridiosis and Colabacillosis (caused by *E.coli*) that attack the abdominal region (stomach and intestines) of humans and animals.

The composition is prepared in powder form, in syrup form or in paste form.

Ingredient	Veterinary use	Medical (human) use
$CaCO_3$	94%	—
Lactose	—	91%
Tannin	1%	8%
Origanum vulgare ssp. *hirtum*	5%	8%

The preparation is carried out in a vacuum mixer, where 50 wt % of the total quantity of tannin and oregano essential oil are added to $CaCO_3$ or lactose (depending on the desired use (for human or animal) and the blend is mixed at 200 rpm for 20 minutes. Subsequently the rest of the tannin and oil are added to the blend and are mixed for an additional 45 minutes.

The powder thus produced is packed in packets of 100, 250, 500, 1000, 2000 g, made of plastic layered aluminum foil and in sacks of 25 or 50 kg for the powder intended for veterinary use, while the powder for medical use is filled in 500 mg capsules.

b) Syrup Form

As mentioned above, the pharmaceutical composition for the treatment of Salmonellasis, Staphylococciasis, Pasteuridiosis and Colabacillosis (caused by *E.coli*) can also be in syrup form. The essential starting materials and corresponding amounts for the preparation of said syrups are as follows:

Ingredient	Veterinary use	Medical (human) use
Polyethylene glycol	92.5%	94.5%
Origanum vulgare ssp. *hirtum*	5%	3%
Tannin	1%	1%
Glycerine monostearate	1.5%	1.5%

The process for preparing the above syrup is carried out in a colloidal mixer, where the polyethylene glycol is charged first and heated at 55° C. for 5 minutes. Subsequently, the *Origanum vulgare* ssp. *hirtum* oil, the tannin and the glycerine monostearate are added and mixed together at the same temperature for 15 minutes at a speed of 300 rpm. The mixture is allowed to cool for one hour and then is mixed again, without heating, for 30 minutes at 300 rpm.

The syrup thus prepared is filled in dark coloured glass flasks of 120 ml and the flasks intended for animal use are provided with a suitable fitter to facilitate administration to animals.

c) Paste Form

According to the same method, but with slight variation of the levels of the various ingredients, a paste form is produced, intended for veterinary use only.

Ingredients	
Polyethylene glycol	74%
Origanum vulgare ssp. *hirtum*	5%
Tannin	1%
Glycerine monostearate	20%

The paste in filled in 100 ml tubes that are provided with a special nozzle for administration to animals.

Example 2

One additional form of powder is that intended for the prevention and treatment of coccidiosis in poultry, caused by the germs of the Eimeria group (*E. tenella, E. acervulina, E. colhici, E. duodenalis, E. mitri, E. fasiani* and the like). For the preparation of this powder the essential ingradients are used in the following levels:

Ingredient	Veterinary use (poultry)
$CaCO_3$	90%
Origanum vulgare ssp. *hirtum*	5%
Glycerine monostearate	1%

The preparation process is the same as above with the difference that tannin is not used.

For the prevention and treatment of coccidiosis, a solution form can also be prepared, with the following essential ingredients in the corresponding levels:

Ingredients	
Origanum vulgare ssp. *hirtum*	5%
Emulgator 484	3%
Propylene glycol	10%
Distilled water	82%

The preparation is carried out in a colloidal mixer, where the Emulgator 484 is charged first and the essential oil is added and mixed at 200 rpm for 10 minutes. Next, the propylene glycol is added and mixed for an additional 10 minutes and finally the water is added and mixed at the same speed for an additional 10 minutes. The solution thus prepared is filled in 1 liter bottles and is useful in the treatment of coccidiosis in poultry.

The same solution is useful for the purification and disinfection of drinking water. The quantities which provide sufficient bacteriostatic activity without imparting a disagreeable odour are in the range of 10 to 20 ml of the above solution per cubic meter water.

Example 3

Among the pharmaceutical compositions of the present invention, one is intended for the fungal infections of the human and animal skin. The composition may be in the form of tincture or ointment.

a) Tincture form

Ingredient	Veterinary use	Medical (human) use
Paraffinum liquidum	95%	—
Propylene glycol	—	95%
Origanum vulgare ssp. *hirtum*	5%	5%

Depending on the desired end use, propylene glycol or paraffinum liquidum is heated in a vacuum mixer at 55° C. and, at the same temperature, the *Origanum vulgare* ssp. *hirtum* oil is added and mixed for 20 minutes at 450 rpm.

The composition is filled in 10 ml dark coloured vials provided with suitable plastic applicator for skin administration.

b) Ointment Form

For the treatment of dermal fungal infections, the ointment form of the present invention has the following composition:

Ingredient	Veterinary use	Medical (human) use
Paraffinum liquidum	25%	20%
Origanum vulgare ssp. *hirtum*	5%	5%
Vaselinum album	70%	75%

The preparation takes place in a colloidal mixer, where the paraffinum liquidum is charged first and heated at 55° C. Next, the origanum oil is added and mixed for 15 minutes at 300 rpm. Vaseline is preheated at 65° C. and then added to the mixture. The total is mixed for 45 minutes at 600 rpm. Before the mixture is cooled, it is charged in a suitable funnel for filling tubes.

Example 4

For the treatment of breast inflammations in animals (mastitis caused by Staphylococcus, Streptococcus as well as chronic mastitis) there is a process for the preparation of a composition comprising the following ingredients:

Ingredients	
Paraffinum liquidum	45%
Origanum vulgare ssp. *hirtum*	5%
Vaselinum album	50%

The process comprises heating the paraffinum liquidum at 55° C. in a colloidal mixer, where the *Origanum vulgare* ssp. *hirtum* oil is subsequently added and mixed for 15 minutes at 300 rpm. The vaseline, preheated at 65° C., is introduced to the mixture and the total is mixed for 45 minutes at 600 rpm.

The mixture, as it is, is filled in 10 ml plastic syringes fitted with stopper and suitable nozzle for administration to the breast duct.

Example 5

For the treatment of infections and inflammations caused by various germs and fungi in the vagina and uterus of women and female animals, there is a process for the preparation of a composition in the form of vaginal suppositories comprising the following ingredients:

Ingredients	
Origanum vulgare ssp. *hirtum*	3%
Hygroscopic carrier	97%

The process comprises mixing in a colloidal mixer, without heating, the *Origanum vulgare* ssp. *hirtum* oil and the hygroscopic carrier for 30 minutes at 300 rpm. In the same vat, the mixture is placed in a suppository forming press, which forms suppositories by pressing the mixture in heated moulds. The produced suppositories of 5, 10 or 20 g are packed in airtight package.

Example 6

For the treatment of aural infections including otitis caused by various germs in humans and animals, there is a process for the preparation of a composition in the form of drops comprising the following ingredients:

Ingredients	
Origanum vulgare ssp. *hirtum*	3%
Paraffinum liquidum	97%

The process comprises heating in a colloidal mixer the paraffinum liquidum at 55° C., adding the *Origanum vulgare* ssp. *hirtum* oil and mixing the blend for 20 minutes at 300 rpm. The product is filled in dark coloured vials with suitable dropper tube.

Example 7

A pharmaceutical composition for the treatment of inflammations caused by various germs in injuries of humans and animals, is in the form of spray or powder.

a) Powder Form

For the powder form, the ingredients are used in the following amounts:

Ingredients	Veterinary use	Medical (human) use
Origanum vulgare ssp. *hirtum*	3%	5%
Starch	97%	—
Baby powder Neutral	—	95%

Equal quantities of powder (starch or baby powder Neutral, according to the end use) and *Origanum vulgare* ssp. *hirtum* oil are charged in a vacuum blender and blended for 10 minutes at 60 rpm. Then half of the remaining powder is added and blended for 20 minutes at the same speed. Finally, the remaining powder is added and blended at 300 rpm for 30 minutes. The powder obtained is filled in plastic layered aluminum bags of 100 g.

b) Spray Form

In order to prepare the spray form for veterinary use only the ingredients listed below are used in the following amounts:

Ingredients	
Origanum vulgare ssp. *hirtum*	3%
Carrier	97%

The total quantities of the ingredients are mixed in a colloidal mixer at 600 rpm for 30 minutes and the blend is filled in 200 ml flasks equipped with a suitable spraying pump.

Example 8

A pharmaceutical composition directed to the treatment of inflammations of the lungs and kidneys of animals (pneumonia, nephritis) is in form of an injectable solution for intramuscular administration to animals, comprising the following ingredients:

ingredients	
Origanum vulgare ssp. *hirtum*	1.5%
Polyethylene glycol	98.5%

Polyethylene glycol is heated in a colloidal mixer at 55° C., the origanum hyrtum oil is added to the mixer and the total is mixed at 600 rpm for 30 minutes. The solution is filled in 120 ml airtight flasks fitted with a suitable rubber stopper.

Example 9

Another application is a pharmaceutical composition for the treatment of ophthalmic inflammations that cause conjunctivitis in humans and animals, comprising the following components:

Ingredients	Veterinary use	Medical (human) use
Paraffinum liquidum	37%	—
Origanum vulgare ssp. *hirtum*	3%	2%
Vaselinum album	60%	98%

The process is carried out in a colloidal mixer, where vaseline preheated to 65° C. is charged followed by the remaining ingredients. The blend is mixed at 300 rpm for 30 minutes. The product is packed in tubes of 100 and 20 g, depending on the desired use (veterinary or medical).

Pharmaceutical compositions may be prepared by application of the same methods and using the same ingredients as described in Examples 1 to 9 except for the substitution of *Origanum vulgare* ssp. *hirtum* by *Origanum heracleoticum* (referred to above as Specimen 4) as well as by any of the other specimens of Table 1.

A mixture of the essential oils of *Origanum vulgare* ssp. *hirtum* (Specimen 2) and *Origanum heracleoticum* (Specimen 5) (as obtained by steam distillation) in a 1:1 weight ratio, comprising carvacrol and thymol in levels of 82.0% and 1.5% respectively (carvacrol:thymol ratio=54, 7:1), may also be used in the same amounts as *Origanum vulgare* ssp. *hirtum* oil in Examples 1 to 9 for the preparation of the analogous pharmaceutical compositions.

I claim:

1. An antimicrobial pharmaceutical composition comprising an antimicrobial-effective amount of an essential oil obtained from *Origanum vulgare* ssp. *hirtum* containing thymol and carvacrol as its main ingredients, and a pharmaceutically acceptable carrier, wherein

(a) the total amount of thymol and carvacrol in said essential oil is at least 55%, by weight, of said essential oil, and

(b) the ratio of carvacrol to thymol is at least 10:1.

2. The pharmaceutical composition according to claim 1, wherein said ratio of carvacrol to thymol is in the range of 30:1 to 150:1 and said essential oil is obtained by steam distillation of said *Origanum vulgare* ssp. *hirtum*.

3. The pharmaceutical composition according to claim 1, wherein said essential oil is obtained by aqueous alcoholic extraction of said *Origanum vulgare* ssp. *hirtum*.

4. The pharmaceutical composition according to claim 3 for use in the treatment of diseases caused by pathogenic microorganisms of the abdominal tract.

5. The pharmaceutical composition according to claim 1 for use in the prevention and treatment of coccidiosis in poultry.

6. The pharmaceutical composition according to claim 1 for use in the treatment of diseases selected from the group consisting of:

a) chronic mastitis or mastitis caused by Staphylococcus or Streptococcus,

b) dermal fungal infections and inflammations including infections and inflammations in a vagina or uterus,

c) aural infections,

d) ophthalmic inflammations,

e) inflammations of lungs or pneumonia, and

f) inflammations of kidneys or nephritis.

7. The pharmaceutical composition according to claim 1 for use in disinfecting water.

8. The composition according to claim 1, wherein the total amount of thymol and carvacrol in said essential oil is at least 70%, by weight, of said essential oil.

9. A method of preparing an antimicrobial pharmaceutical composition comprising obtaining the essential oil of *Origanum vulgare* ssp. *hirtum* by steam distillation and mixing an antimicrobial-effective amount of said essential oil with a pharmaceutically acceptable carrier, wherein

(a) the total amount of thymol and carvacrol in said essential oil is at least 55%, by weight, of said essential oil, and

(b) the ratio of carvacrol to thymol is at least 10:1.

10. The method according to claim 9, wherein said ratio of carvacrol to thymol in said essential oil is in the range of 30:1 to 150:1.

11. The method according to claim 9, further comprising obtaining the essential oil of *Origanum vulgare* ssp. *hirtum* by aqueous alcoholic extraction and admixing it with the essential oil obtained by steam distillation and the pharmaceutically acceptable carrier.

12. A method of treating or preventing coccidiosis in poultry comprising the step of administering to poultry an effective amount of the anitmicrobial pharmaceutical composition according to claim 9.

* * * * *

US006322825B1

(12) **United States Patent**
Ninkov

(10) **Patent No.: US 6,322,825 B1**
(45) **Date of Patent: Nov. 27, 2001**

(54) **COMPOSITIONS CONTAINING THYMOL AND CARVACROL AND METHODS OF TREATING GASTROINTESTINAL INFECTIONS WITH THE COMPOSITIONS**

(75) Inventor: **Dusan Ninkov**, Amstelveen (NL)

(73) Assignee: **Ropapharm B.V.**, Zaandam (NL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/356,499**

(22) Filed: **Jul. 19, 1999**

Related U.S. Application Data

(63) Continuation of application No. 08/875,991, filed as application No. PCT/NL96/00210 on May 24, 1996, now abandoned.

(30) **Foreign Application Priority Data**

May 26, 1995 (MK) 950075
May 26, 1995 (MK) 950076

(51) **Int. Cl.**[7] **A61K 35/78**; C07C 39/06

(52) **U.S. Cl.** **424/745**; 424/725; 424/747; 424/774; 568/781

(58) **Field of Search** 424/195.1, 725, 424/745, 747, 774; 568/781

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,939,260		2/1976	Lafon	424/28
4,318,906	*	3/1982	Llopart	424/195.1
5,142,817		9/1992	Rolf	47/24
5,252,344		10/1993	Shi	424/682
5,591,435	*	1/1997	Vaccarello-Dunkel et al.	424/195.1
5,665,781	*	9/1997	Warren et al.	514/703
6,106,838	*	8/2000	Nitsas	424/195.1

FOREIGN PATENT DOCUMENTS

WO 97/01348 1/1997 (WO) .

OTHER PUBLICATIONS

Lawless, "The Illustrated Encyclopedia of Essential Oils: The Complete Guide to the Use of Oils in Aromatheraphy and Herbalism", Barnes & Noble Books, pp. 188, 227 and 228.

"Remington's Pharmaceutical Sciences", 16th Edition, pp. 1256–1267.

The Merck Index, 12th Edition, pp. 308 and 1604.

K.D. Gunther et al., "The Effect of Etheric Oil From Origanum Vulgaris (Ropadiar) in the Feed Ration of Weaned Pigs on Their Daily Need Intake, Daily Gains and Food Utilization", Oral Presentations, Jul. 5 to 9, 1998.

"Ropadiar Emulsion Clinical Examination Report", College of Veterinary Medicaine, China Agricultural University.

"Registered License for Animal Health Product", Apr. 1988.

D. Hoffman, The Herbal Handbook, A User's Guide to Medical Herbalism, Healing Arts Press, Rochester, Vt, p. 128, 1988.*

Guerin et al., Ann. Pharmaceutiques Francaises, 43:77–87, 1985.*

Lawless, The Illustrated Encyclopedia Of Essential Oils, The Complete Guide to the Use of Oils in Aromatheraphy and Herbalism, Element Books Inc., Rockport MA, pp. 141, 149, 175, and 212, 1995.*

The Merck Manual of Diagnosis and Theraphy, 16th Edition, Eds. Berkow, Fletcher, and Beers, Merck Research Laboratories, Merck & Co., Inc., Rahway, NJ, pp. 812–821, 1992.*

* cited by examiner

Primary Examiner—Christopher R. Tate
Assistant Examiner—Janet M. Kerr
(74) *Attorney, Agent, or Firm*—Young & Thompson

(57) **ABSTRACT**

The invention relates to pharmaceutical compounds which are based on the anti-flammatory properties of etheric oils selected from the group consisting of *Origanum vulgaris, Thymus vulgaris, Mentha piperita, Thymus serpilum, Saturea hortensis, Saturea montana, Saturea subricata, Carum corticum, Thymus zugis, Ocimum gratisimum, Moranda pungtata, Mosla japanoica* and *Salvia officinalis.* Preferably the etheric oils, obtained at the distillation of *Origanum vulgaris, Thymus vulgaris* and/or *Mentha piperita* are used. Such pharmaceutical compounds, compared to synthetic sulfonamids, antibiotics and cortisones do not create biorecidives in the human body as well as in animal meat and milk and milk and do not contribute to the resistance of microorganism against pharmaceutical compositions in general. The composition according to the invention can be used in the treatment of colibacillosis, dermatomycosis, lice, perspiration and fungas on feet, dermatitis, acne and of veterinary diseases such as coccidiosis and mastitis.

10 Claims, No Drawings

COMPOSITIONS CONTAINING THYMOL AND CARVACROL AND METHODS OF TREATING GASTROINTESTINAL INFECTIONS WITH THE COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 08/875,991 filed Jan. 26, 1998 abandoned, which is a 371 of PCT/NL96/00210 filed May 26, 1996.

DESCRIPTION

1. Field of Technology

The invention relates to pharmaceutical compositions, comprising etheric oils extracted from specific plants, a process for preparing such pharmaceutical compositions, as well as to the application of said compositions in the human and veterinary medical field.

2. Technical Problems Solved With This Invention

One of the technical problems that is solved with this invention is the utilization of various types of human medicaments on the basis of active natural components that successfully replace prior art medicaments based on sulfonamids, antibiotics, cortisones etc., whereby the new products according to the invention:

1. do not create biorecidives in the human body;
2. do not generate resistance of microorganisms;
3. are ecologically unsuspected; and
4. are not toxic, mutagenic or teratogenic.

Another problem that is solved with this invention is the utilization of various types of veterinary medicaments on the basis of natural components, that successfully replace prior art products based on sulfonamids, antibiotics etc.. The application of the active substances according to the invention eliminates many unwanted effects caused by the prior art products like sulfonmaids and antibiotics, such as:

1. the presence of biorecidives in the meat and milk of animals treated with such products; and
2. the resistance of microorganisms-bacteria against the prior art products.

DESCRIPTION OF THE PRIOR ART

In the prior art known medicaments for curing human diseases like: cholera, colibacillosis, dermatomycosis, inflammation of the oral mucosa and pharynx, fungicidal and bacteriological infections, inflammation of the mucous membrane of the vagina, colitis, various etiologies, the treatment of festered wounds, are based on active substances that have an antibiotic, sulfonmaid or hormonal corticoidal origin.

The tendencies of modern science are to substitute a part of the medicaments based on such origins by ecologically healthy drugs, which are much easier for the human organism to bear and have a much more beneficial influence on human health.

The same counts for veterinary medicaments based on antibiotics, sulfonamids and even hormones for treating animal diseases such as cholera, different types of colibacillosis, dermatomycosis, inflammation of the udder, inflammation of the vagina and uterus, various etiologies and coccidiosis. In order to avoid the unwanted consequences mentioned above, it is necessary to have ecologically healthy and unsuspected products for treating these veterinary diseases.

More in particular a great deal of the bacteria that cause the abovementioned diseases have developed a form of resistance against the prior art medicaments, so the products according to the invention resolve the problems of treating the diseases listed above, as well as various types of diarrhea, inflammation of the abdomen, gastritis, inflammation of the oral mucosa, inflammation of the ear, conjunctivitis, etc.

DESCRIPTION OF THE SOLUTION TO THE ABOVE-DESCRIBED PROBLEM

The primary component which is applied in the pharmaceutical compositions according to the invention is etheric oil obtained from any of the following plants: *Origanum vulgaris, Thymus vulgaris, Mentha piperita, Thymus serpilum, Saturea hortensis, Saturea montana, Saturea subricata, Carum corticum, Thymus zugis, Ocimum gratisimu, Moranda pungtata, Mosla japanoica* and *Salvia officinalis.* Preferably the etheric oil is obtained from any of the following plants: *Origanum vulgaris, Thymus vulgaris* and *Mentha piperita.* Most preferably the etheric oil is at least obtained from *Origanum vulgaris* and optionally from *Thymus vulgaris.*

In case of veterinary medicaments it is also possible to use synthetic thymol having the chemical name isopropylcresol. Further the compound tannin may be used in veterinary compositions. This tannin can be recovered by extracting the residue of the leaves and blossoms of the *Origanum vulgaris* plants obtained after the etheric oil-distillation (see below).

The pharmaceutical compositions according to the invention may comprise a pharmaceutically acceptable carrier, preferably of natural origin. Representatives of such carriers are generally known in the human and veterinary pharmaceutical field. Examples of such carriers are lactose, honey, laurel, vaseline, paraffin, starch products, calcium carbonate, etc.

The pharmaceutical compositions may have any form suitable for its application, for instance the form of a capsule, syrup, tincture, ointment, powder, emulsion, paste, etc.

The content of active agent in the pharmaceutical compositions according to the invention, which in fact does also depend on its pharmaceutical use, may vary between wide limits. Preferably the active agent is present in an amount of 1–15% by weight, most preferably 4–10% by weight, calculated on the total weight of the pharmaceutical composition.

In addition to the active agent according to the invention also other active substances, preferably of natural origin, can be used. Such substances may have bacteriological, fungicidal, adstrigidic etc, properties.

The following dosage of a pharmaceutical composition, comprising 5% by weight of oil free *Origano vulgaris* and 95% by weight of calcium carbonate in powder form may be applied for mass and individual treatment:

A) Mass treatment:

Preventive dose;

pigs, rabbits, calves:

500 g of powder per ton of feed

chickens, ducks, turkeys:

450 g of powder per ton of feed

Therapeutical dose:

pigs, rabbits, calves:

1000 g of powder per ton of feed

chickens, ducks turkeys:

900 g of powder per ton of feed for 7–10 days of therapy.

B) Individual treatment:

calves, foals:

0.20–0.25 g per kg of body weight

piglets, lambs, kids:

0.10 g per kg of body weight

For illustrating the pharmacetucial activity of etheric oil from *Origanum vulgaris* (origanum oil) the following "antibiogram" of origanum oil is illustrated in Table A:

TABLE A

Microorganisms	Intensity of Effect
Staphylococcus aureus	+++
Treponema hyodisaenteriae	++++
Erysipelothrix insidiosa	+++
Pasteurella multocida	++++
Streptococcus faecalis	+++
Streptococcus agalactiae	+++
Proteus mirabilis	+++
Proteus vulgaris	+++
Proteus rettgeri	+++
Escherichia coli	++++
Vibrio coli	++++
Salmonella spp.	++++
Streptococcus pyogenes anim.C	+++
Klebsiella pneumoniae	+++
Enterobacter aerogenes	+++
Corynebacterium pyogenes	+++
Streptococcus uberis	+++
Candida spp.	+++
Pseudomonas aeruginosa	+++
Mycobacterium tuberculosis	++++
Aspergilus spp.	++++
Mucor spp.	+++
Cryptosporidiae spp	++++
Eimeria spp. (coccidiosis)	++++

0 resistant;
+ relatively sensitive;
++ moderately sensitive;
+++ very sensitive;
++++ extremely sensitive.

In view of the data in the above Table A it is stated that diseases, caused by the microorganisms in Table A can be cured by pharmaceutical preparations based on origanum oil as active component.

In view of the above, the pharmaceutical compositions are particularly used for prevention and treatment of gastrointestinal infections in humans and particularly in animals, which are caused by the bacteria, fungi etc.; see in this respect the enumeration below:

Pigs: *E. Coli*, Salmonella spp., swine dysentery, Pasteurella spp.

Poultry: (chickens, ducks turkeys): Eimeria spp. (coccidiosis), Salmonella spp., Pasteurella spp.

Rabbits: Eimeria spp. (coccidiosis), Salmonella spp.

Cows: calves-*E. coli*, Pasteurella spp., Salmonella spp.

Lambs: Salmonella spp., Pasteurella spp., *Clostridium perfrigens, E. coli.*

Young goats: (kids): Salmonella spp., Paasteurella spp., Clostridium spp., *E. coli.*

Further to the diseases mentioned above, the pharmaceutical composition according to the invention can be applied for the treatment of:

toxoplasmosis cause by *Toxoplasma gondii* by animals and humans;

internal paristies of dogs like *Toxocara canis, Echinococcus granulosis* etc.:

sarcocystoses by dogs, cattle, poultry and humans;

ascaridosis by pigs and poultry, cause by *Ascaris suum* and *Ascaris golii;*

oxyurosis equi by horses and heteracidosis by poultry;

teniosos by humans and cysticerocis by pigs;

ancylostoma duodenalis and oxyurosis by humans;

rheumatic diseases like arthritis, spondilitis, dyscioathie, and injuring like distorsio, subluxatio etc.;

fungi and insects on plants in the agricultural sector;

demodicosis by dogs;

diarrhea, caused by *Escherichia coli* (*colibacillosos*). Salmonella spp. (*salmonellosis*), Pasteurella spp. (*pasteurellosis*), Streptococcus spp. (*streptococcosis*), *Vibrio* coli (*vibrisis*). *Treponema hyodesynterie* (dysentery-bloody diarrhea), and other kinds of diarrhea by human and animals;

parasitosis caused by Cryptosporidia spp., Ascaridia spp., Toxocara spp., Toxoplasma spp., Atoxoplasmosa;

diseases caused by *Canida albicans,* Aspergilus spp., *Crypotococcus neoformans,* Mucor spp., Fusarium spp., by humans and animals; and

cestodosis by birds and poultry.

The compositions according to the invention, in particular in the form of a powder comprising 5% of origanum vulgare oil, can also be used for the conservation of food for humans and for the conservation of feed for animals and prolongs the storage life of such products.

A process for extracting the etheric oils from the above-mentioned plants, in particular *Origanum vulgaris, Thymus vulgaris* and *Mantha piperita* is carried out by distillation with the help of water vapour (steam).

Firstly, the leaves and blossoms of the plants, which must be dry, are placed in a distiller. In case of human application of the oils obtained, the distillation of the oil from every type of plants is done separately, which means that different types of plants must not be mixed together in the process of distillation.

The distiller should have two output tubes: one for the oil and one for the water vapour (steam). The dish for the water is placed under the dry parts of the plant (the leaves and blossoms) and heated up to 100° C., preferably under a pressure of about 20 bar as an increased pressure will reduce the distillation time. The water vapour passes through the dry parts of the plant, thereby creating oil drops. The drops of water vapour are lighter than the oil drops, hence flow out the output tube at the bottom of the extractor. The drops of oil flow out the output tube for the oil and into the dish intended for the oil. This process is carried out for 3 hours. The yield is 3–6 kg of oil from 100 kg of dry plants. In general the extracted oil contains a certain percentage of thymol and carvacrol: approx. 3% of thymol and 60–70% of carvacrol.

After the above-described distillation, the residue of the leaves and blossoms is used to extract tannin, which can be added to, in particular, veterinary pharmaceutical compositions.

More in particular the procedure for obtaining etheric oils from *Origanum vulgaris* plants consists of four phases:

Phase 1: Selection

On the basis of the existing types of *Origanum vulgaris* plants it has been possible to obtain seeds of *Origanum vulgaris* plants having about 91% active material: 86–88% carvacrol and 3–5% thymol. The remaining 8–10% comprises the following components: linalol, barneol, cimen and some other less important components.

Phase 2: Production of the plants

The seeds obtained in the way according to phase 1 are planted in fine loose soil, preferably in a sub-tropical climate. During this process all agro-technical measure are applied, like watering and artificial fertilizing.

From the already grown plants, only their leaves and blossoms should be used. The reaping should be undertaken while the plants are in blossom early in the morning or late in the evening hours.

Phase 3: Drying of the leaves and blossoms

The drying process is performed in special rooms, i.e. drying houses. The already harvested leaves and blossoms should not be exposed to direct sunlight since any exposure to sunlight significantly decreases the percentage of active material.

The leaves and blossoms are arranged in layers of 20–25 cm thick. During the first three days, these layers should be turned up-side-down four times a day, either manually or mechanically, so that this drying process is proceeded in an uniform way.

These drying houses are constructed in such a way, that the air is able to circulate freely all the time.

The drying process lasts about 7–8 days.

Phase 4: Production of the oil

From the dried leaves and blossoms of the plants, a distillation of the oil is carried out on the basis of a classic steam distillation. Out of 100 kilos of dried leaves and blossoms 5–7 kilos of oil are obtained.

After the distillation step according to which the oil has been obtained, the following step is carried out:

the oil is heated at 187° C., during which process, again performed in the distillator, the substances, which are of no importance are evaporated. The remnants are the important substances: carvacrol 86–88%; thymol 3–5% and in minor quantities: pinene, barneol, linalol etc.

After this redistillation process, the oil is left to cool to room temperature and is then packed in hermetically closed vessels made of aluminum or dark glass.

The invention will be elucidated by the following series of examples, i.e. (A) examples concerning the compositions and preparation of human pharmaceutical medicaments, (B) examples concerning the compositions and preparation of veterinary pharmaceutical medicaments. (C) examples concerning the activity of pharmaceutical medicaments according to the invention and (D) examples concerning the safety of pharmaceutical medicaments according to the invention. The percentage is expressed as percentage by weight unless otherwise indicated.

A) COMPOSITION AND PREPARATION OF HUMAN PHARMACEUTICAL MEDICAMENTS

EXAMPLE 1

Medicaments for the treatment of colibacillosis, salmonellosis, pasteurellosis, vibriosis and cholera: the diseases are caused by *Escherichia coli, Salmonella typhimuxium, Vibrio fetus* and *Pasteurella multocida.*

1.1 Procedure for making capsules.

The integral parts of the substances from which the medicament is prepared are:

lactose	90–92%
Origanum vulgaris oil	4–6%
Thymus vulgaris oil	2–4%
Mentha piperita oil	0.5–1.5%

Firstly, about 30% of the lactose and the total amount of oils are put in a vacuum mixer. The mixture is mixed for five minutes at a speed of 200 rotations per minute. Then the rest of the lactose is added and everything is mixed together for another 10 minutes at the above-mentioned speed. Finally, the powder is packed into capsules.

1.2 Procedure for making a syrup.

The integral parts of the substances from which the medicament is prepared are:

honey as the basic element	92–94%
Origanum vulgaris oil	2–4%
Thymus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%

30% of the overall amount of honey in a liquid form is placed in a vacuum mixer and the whole amount of oils is added. The mixture is mixed for 15 minutes at a speed of 200 rotations per minute. Then the rest of the honey is added and mixed for another 15 minutes at the above-mentioned speed. The resulting product is a syrup in liquid form, which can be packed as soon as it is cooled down.

EXAMPLE 2

Medicaments for the treatment of dermatomycosis: dermatomycosis may be caused by Trychophiton sp. and Microspora sp.

2.1 Process for obtaining a tincture.

The integral parts of the substances used to prepare the medicament are:

polyethylene glycol	72–74%
laurel	19–21%
Origanum vulgaris oil	3–5%
Thysus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%

Approximately 30% of the overall amount of polyethylene glycol is heated in a vacuum mixer up to 55° C. The total quanity of oils is added and mixed at a speed of 200 rotations per minute. At the end the rest of the polyethylene glycol and the laurel are added and this is mixed for five more minutes at the above-mentioned speed. After it is cooled down, the product is ready to be packed.

2.2 Procedure for making an ointment.

The integral parts of the substances used to make the ointment are:

vaselin album	67–69%
parafinum liquidum	24–26%
Origanum vulgaris oil	3–5%

-continued

Thymus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%

30% of the vaseline album is melted in a vacuum mixer at a temperature of 45° C. The oils are added and everything is mixed together for 10 minutes at a speed of 250 rotations per minute. Then, the rest of the vaselin and the parafinum liquidum are added. Everything is mixed for another 10 minutes at the above-mentioned temperature and packed after it is cooled down.

EXAMPLE 3

Medicaments for the treatment of colpitus (for women): colpitus may be caused by *Trychomonos genetalis.*

The substances used to prepare this medicament are:

starch dextrose	51–53%
hygroscopic carrier	19–21%
kolloid	19–21%
Origanum vulgaris oil	2–4%
Thymus vulgaris oil	2–14%
Klamath weed oil	1–3%

The process is as follows: the starch dextrose and the neutral hygroscopic carrier are placed in a vacuum mixer. The prescribed quantities of oils are added to this mixture and everything is blended at a speed of 200 rotations per minute. At the end, the kolloid is added, everything is mixed once more, and packed.

EXAMPLE 4

Repellent for lice and other types of skin insects: e.g. mosquitos and flies.

The substances used to obtain the product are:

Laurel	91–93%
Origanum vulgaris oil	4–6%
Thymus vulgaris oil	2–4%

All the components listed above are put in a vacuum mixer. They are mixed together at a speed of 200 rotations per minute, for 10 minutes, after which the mixture is packed. The advantage of this product over the chemical varieties based on lindane is that the medicaments according to the invention are completely non-toxic for humans and animals.

EXAMPLE 5

Product for the prevention of foot perspiration and of the presence of fungus on feet: Trychophiton sp. and Microspora sp.

The substances that are used in the procedure for preparing these medicaments are:

calcium carbonate	91–93%
Origanum vulgaris oil	4–6%
Thymus vulgaris oil	2–4%

30% of the calcium carbonate and the total amount of the oils are put in a vacuum mixer. The mixture is mixed for 10 minutes at a speed of 200 rotations per minute and subsequently the rest of the calcium carbonate is added. The mixing is continued until a powder is obtained.

EXAMPLE 6

Product for the extermination of insects and other pest: e.g. mosquitos and flies.

The substances that are used in the process of preparing this product are:

calcium carbonate	91–93%
Origanum vulgaris oil	4–6%
Thymus vulgaris oil	2–4%

30% of the calcium carbonate together with the entire amount of oil is put in a vacuum mixer. The mixture is blended for 10 minutes at a speed of 200 rotations per minute. Subsequently, the rest of the calcium carbonate is added and the mixing is continued until a fine powder is obtained.

EXAMPLE 7

Medicament for the treatment of dermatitis, acne and other inflammations of the skin on the face.

The substances from which the medicament are prepared are:

laurel	72–74%
ethanol	19–21%
Origanum vulgaris oil	3–3%
Thymus vulgaris oil	2–4%

Some 30% of the laurel together with the whole amount of oils is put in the vacuum mixer. It is mixed for 10 minutes at a speed of 200 rotations per minute. Subsequently, the rest of the laurel and the ethanol are added and the product is mixed and packed in dark glass bottles.

EXAMPLE 8

Medicament for Wounds

The substances used in the process for preparing the medicament are:

neutral medical powder	94–96%
Origanum vulargis oil	2–4%
Thymus vulgaris oil	1–3%

30% of the neutral medical powder and the total amount of oils are put in the vacuum mixer and mixed together for

10 minutes at a speed of 200 rotations per minute. Subsequently, the rest of the neutral medical powder is added and mixed. The product is packed in small bags or aluminum tubes under pressure.

COMPOSITIONS AND PREPARATION OF VETERINARY PHARMACEUTICAL MEDICAMENTS

EXAMPLE 9

Medicaments for the treatment of colibacillosos and gastroenteric diseases in animals: colibacillosis may be caused by *Escherichia coli* and other species: Salmonella, Pasteurella, Vibrio, Treponema, Hiodysenterie and Cryptosporidiosae sp.

9.1 Procedure for making a powder.

The substances which are used in the procedure for making a powder for the treatment of colibacillosis are:

calcium carbonate	91–93%
Origanum vulgaris oil	3–5%
Thymus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%
tannin	0.5–1.5%

30% of the total quantity of calcium carbonate is put in a turbo vacuum mixer and the entire amount of etheric oils is gradually added. The total product is mixed together at a speed of 250 rotations per minute for 10 minutes. The etheric oils are mixed together directly before being poured in the mixer. After the mixing time of 10 minutes, the rest of the calcium carbonate is added and mixed at the above-mentioned speed for another 5 minutes. The powder is then ready to be packed.

9.2 Procedure for making an emulsion.

In one case the substances which are used in the procedure for preparing the product are:

polyethylene glycol	89.5–91.5%
Origanum vulgaris oil	3–5%
Thymus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%
tannin	0.5–1.5%
glycerol monostearate	1–2%

and in the second case these substances are:

laurel oil	91–93%
Origanum vulgaris oil	3–5%
Thymus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%
tannin	0.5–1.5%

The procedure is in both cases the same. Firstly, 30% of the basic substance is placed in a turbo vacuum mixer (in the first case, the polyethylene glycol, and in the second case the laurel oil) and the mixture of oils is added. Everything is mixed together for a period of 5 minutes at a speed of 200 rotations per minute. After this is done, the rest of the basic substance is added. In the first case the glycerol monostearate is added as well. It is mixed for another 10 minutes with the same intensity. At the end it is packed in dark glass bottles.

9.3 Procedure for the production of capsules.

The substances which are used in the procedure for preparing the product are:

lactose	91–93%
Origanum vulgaris oil	3–5%
Thymus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%
tannin	0.5–1.5%

Firstly, the three types of oils are mixed in the given percentage. The mixture is then blended together in a mixer with 30% of the lactose at a speed of 200 rotations per minute for a period of 10 minutes. The rest of the lactose is added and mixed again. The mixture is then put in capsules.

9.4 Procedure for making a paste.

The elements which are used in the procedure are:

vaselinum album	69–71%
Origanum vulgaris oil	3–5%
Thymus vulgaris oil	1–3%
Mentha piperita oil	0.5–1.5%
tannin	0.5–1.5%
parafinum liquidum	21–23%

The procedure for making the paste goes as follows: the vaselinum album is placed in the mixer together with 20% of the total contents and heated up to a temperature of 40° C. The heated mass is then added to the mixture of above-mentioned quantities of oil and the product is mixed together for 5 minutes at a speed of 100 rotations per minute. At the end the parafinum liquidum is added and the product is packed.

EXAMPLE 10

Product for the Treatment and Prevention of Coccidiosis in Livestock

Coccidiosis may be caused by *Eimeria tenella, Eimeria phasani, Eimeria mecarriz, Eimeria duodenalis, Eimeria acervulina, Eimeria colchici, Eimeria maxima, Eimeria praecox, Eimeria brunetti, Eimeria Hagani, Eimeria mitis, Eimeria mivoti.*

The substances which are used in the preparation of the product are:

calcium carbonate	92–94%
Origanum vulgaris oil	3–5%
Thymus vulgaris oil	1–3%
Menths piperita oil	0.5–1.5%

20% of the calcium carbonate is put in a turbo vacuum mixer and the total amount of oils is added after being mixed beforehand. The product is blended together for 10 minutes at 200 rotations per minute. Then the rest of the calcium carbonate is added and mixed again for another 10 minutes. Finally the product is packed.

EXAMPLE 11

Product for the Treatment of Mastitis

Mastitis may be caused by *Streptococcus uberis, Staphilococcus aureus, Escherichia coli, Cryptococcus neoformans, Candida albicans, Spheroforus necroforus, Streptococcus agalactie.*

The substances which are used in the procedure for the preparation of the product are:

vaselinum album	92–94%
Parafinum liquidum	22–24%
Origanum vulgaris oil	3–5%
Mentha piperita oil	0.5–1.5%

20% of the vaselinum album is put in a mixer that is previously heated at 45° C. After the substance is melted the mixture of the given quantities of oils is added and mixed for 5 minutes at a speed of 200 rotations per minute. The rest of the vaselinum album and all of the parafinum liquidum are added as the product tools down. Then, the product is packed in appropriate syringes.

EXAMPLE 12

Product Having Anti-Dermatitis Properties

Dermatitis can be caused by Trychophiron sp. and Microspora sp.

The substances used in the procedure for preparing the product are:

vaselinum album	69–71%
parafinum liquidum	21–23%
Origarium vulgaris oil	3–5%
Thymus vulgeris oil	2–4%
Mentha piperita oil	0.5–1.5%

20% of the vaselinum album is put in a mixer which is previously heated at 45° C. The oils are added and the product is mixed for 10 minutes at a speed of 50 rotations per minute. Then the rest of the vaselinum album and the parafinum liquidum are added. Finally the mass is cooled down and packed in appropriate tubes.

EXAMPLE 13

Products for the Treatment of Animal Wounds

The substances used in the procedure for preparing the product are:

medical powder	92–94%
Origanum vulgaris oil	4–6%
Thymus vulgaris oil	0.5–1.5%
Mentha piperita oil	0.5–1.5%

20% of the medical powder is put in a vacuum mixer and mixed together with the oils which are mixed beforehand. The mixture is blended together for 10 minutes at a speed of 200 rotations per minute. Then the rest of the medical powder is added and mixed another 5 minutes at a speed of 150 rotations per minute. Finally the powder is packed in bags or bottles under pressure.

c) EXAMPLES CONCERNING THE ACTIVITY OF VETERINARY PHARMACEUTICAL MEDICAMENTS ACCORDING TO THE INVENTION

EXAMPLE 14

Introduction

Coccidiosis belongs to the most common acute diseases of the modern poultry upbringing. The disease is caused by monoxenous intercellular parasites from Eimeria species, from which pathogenecity depends the state of the disease and mortality level. Aiming for prevention and eradication of the coccidia today many chemotherapeutics are being used, but there appears to be a problem with the resistance of these parasites to them, especially if used inadequately and without changes. For this reason, the present invention provides immune protection of poultry (vaccines) by means of a preparation according to the invention composed of 5% by weight of *Origanum vulgaris* oil and 95% by weight of $CaCO_3$ and indicated below as "preparation A".

Materials and Methods of Work

The tests have been done on 75 chickens of the "Hybro" species. The chickens were grown on one mini-farm, with floor system and fed by standard concentrate "ad libidum". At the beginning of the test they have been separated in three groups of 25 animals each.

Group A: In 25 chickens one day old, preparation A has been added to the food as a prevention dose, which is 0.25 gr/kg of food for 7 days. After the 7th day, there was found an infection by oocysts of *E. Tenella* and *E. Acervulima*. After the infection the chickens were observed up to 28 days with regular examination of excrement. After the 28the day, the chickens were sacrificed and examined for its parasitology section, together with examination of the excrement.

Group B: Within this group of chickens, 25 chickens were given concentrate without coccistatics and test-preparation A. After the 7th day the infection by oocysts *E. Tenella* and *E. Acervulina* appeared. The same day preparation A was been mixed with the food of the animals in a concentration of 0.5 gr/kg of food. The chickens were fed by food mixed this way for seven days, and after this, were observed followed by regular parasitology examination started at the moment of detection of the infection. After the 28th day, the chickens were sacrificed and examined for its parasitology section, together with examination of the excrement.

Group C: In this group of 25 chickens, animals were fed 7 days with pure food, and after the 7th day there appeared an oocyst of *E. Tenella* and *E. Acervulina* infection. After clinical manifestation of the disease (7th day after infection) their food was mixed with preparation A, in a concentration of 0.5 gr/kg of food. The treatment lasted for 7 days while regular parasitology examinations were performed. 28 day old chickens were sacrificed and examined for its parasitology section, together with examination of the excrement.

The infection of the poultry has been obtained by tetrenic isolates of oocysts of *E. Tenella* and *E. Acervulina*, which after standard application and examination of the sporulation have been given to each individual chicken in doses of 1×10^5 oocysts. The examinations were performed by standard parasitology methods given by Johnson J. and Reid W. M., 1970, Anticoccidial drugs: lesion scorion technique in battery and floar pen experiments with chickens. Ex.Parasitol. 28, 30–36. The examinations were been done the first day after moving the chickens in, and after their infection in two day intervals during the complete test.

The Results of Test and Discussion

By examination done the first day after moving the poultry in, there has not been found a parasite infection in the animals. After the infection the examination was performed in two day intervals, in all three experiment groups. In groups A and B, coccidia was not detected in excrement. In group C the sixth day after infection the clinical symptoms of coccidiosis were detected (diarrhea, characteristic

behaviour . . .) as well as presence of oocysts of coccidia in the excrement. The second day after treatment, the diarrhea stopped but oocysts of coccidia were still found in the excrement. On the fourth day the number of oocysts significantly decreased and during next examination it dropped to a minimum. The clinical picture of the disease stopped after the fifth day of the treatment. No chickens died. After sacrifice of the chickens and parasitology section, together with examination of the excrement were performed by method of Johnson and Reid (1970), loc.cit. In groups A and B they have not been noticed while in group C they were not significantly important (level +1 according to this scale, never more than this).

Based on preliminary examination it can be concluded that the application of the preparation A can be used for coccidiosis of the poultry and have been efficient in preventive and therapeutic effect. Having in mind that the preparation contains 5% ethereal oil of *Origanum vulgaris* flower and leaf, which has a healing effect on the intestinal tract, this is probably the reason for the absence of changes in digestive tract of infected poultry clinically found before the application of the therapy. Within this group, the quick recovery was noticed and they have developed normally. It was noticed that poultry has normally taken the medicated food and that the strong smell of oregano has been absorbed in the food normally and therefore kept unnoticed. The powder is a better form for homogenization in concentration-form for the poultry. During the experiment, the poultry got all food ad libidum, and the micro-climate in the object moved between optimal limits for this kind of production and optimal space was provided for the animals.

Conclusion

Based on the experiment using preparation A for use in the prevention and eradication of coccidiosis in chickens we can conclude the following:

- The preparation in the concentration of 0.25 gr/kg of food can be successfully used in prevention of this disease. The preparation should be used by adding it to the food within 7 days;
- The preparation in the concentration of 0.50 gr/kg of food can be successfully used during the beginning and clinically manifested form of coccidiosis. Therapy should be applied for 7 days;
- The preparation mixes easily with the food. The preparation smells strongly of oregano, but in the food it cannot be tasted. Chickens normally eat food with mixed preparation in it;
- In treated chickens there were no side effects noticed as a consequence of the application of this preparation;
- The preparation can be applied both for preventive or therapeutical use, and also for other diseases of poultry.

EXAMPLE 15

Introduction

As brought up in example 14, coccidiosis belongs to the most common parasitic diseases of poultry, for instance pheasants in intensive upbringing. The most common clinically manifested disease appears in pheasants aged 4–6 weeks, causing significant losses to this production. The coccidiosis prevention and therapy is analogous to the poultry production with the application of identical therapeutics. The problem of the resistance to it is present in this case as well. For these reasons the testing of the preventive and therapeutic effect of the preparation A (defined in example 14) was done and the results show significant efficiency of this preparation used in this test.

Materials and Methods of Work

The tests have been done of pheasants 3–6 weeks old, bearing in mind that coccidiosis in clinically manifested form appears in pheasants 4–6 weeks old. Based on dynamics of the production in the pheasant farm, every group of the birds counts approximately 2000 animals. The experiment has been performed on three groups.

Group A: On pheasants three weeks old, after parasitologic examination, in which no infection of cocci was found, preparation A has been added to the food. For prevention purposes, the preparation has been given in a concentration of 0.25 gr/kg of foods seven days in a row.

Group B: Within this group of pheasants, 24 days old, in obduction has been bound the beginning stage of the cocci infection. The estimation of the infection degree is being observed through the number of cocci and pathological changes in intestines. Determination of the causer-species is based on their morphological characteristics. After diagnosis the therapy doses of preparation A has been given in a concentration of 0.5 gr/kg of foods seven days in a row.

Group C: In this group the spring pheasants 4 weeks old, have clearly shown clinical symptoms of the disease. Characteristic behaviour of the birds and diarrhea were obvious in this group. *Hemoragical enteritis*, which mycosis corks in cecum, was observed. The death of the pheasants was within the limits of 50–60 birds daily. The therapy of preparation A has been applied in a concentration of 0.5 gr/kg of food.

Diagnosis of coccidiosis has been made by examining samples of excrement by standard parasitology methods. The smear of the mocous membrane has been done from the changed areas on intestines of the diseased pheasants. The estimation of extensivity and intensity of the infection has been done by counting oocysts of cocci, and based on the degree of pathological changes. Determination is based on morphological characteristics of the parasites by means of the Norton method (1981).

THE RESULTS OF TESTS AND DISCUSSION

Group A: Coccidiostatic effect of the preparation A has been completely successful. During observation of the animals for three weeks (ending 6th week) there were no cases of the presence of cocci. In obduction there were no parasite elements found (oocysts of cocci). Pheasants have developed normally, and the degree of mortality was below the technological range anticipated for this production (in average 1.1%).

Group B: Within this group of pheasants the beginning infection has been diagnosed, caused by *E. Duodenalis* and *E. Colchici*. The degree of the mortality was within technological limits, but the indication symptoms of the infection have been noticed. After 7-days of therapy the symptoms have completely disappeared and in obduction of dead and sacrificed animals there were no pathological changes in intestines and coccum, characteristic for coccidiosis. The examinations of the smear of mucous membrane have shown that the disease has not developed.

Group C: This group had present coccidiosis in clinical range followed by mortality of 2.5%. Infection has been caused by *E. Duodenalis* and *E. Colchici*. Therapy has been applied for 7 days. During the first three days the mortality has been significantly decreased, while after completed

therapy coccidiosis has been eradicated completely. This has been approved by obduction and parasitology examination of the sacrificed and dead pheasants.

In both groups (B and C) where the infection has been noticed, after therapy there has been an important and significant improvement in condition and health of the birds. Following the development of these birds for the next several weeks, it has been noticed that vitality and development is within optimal range for this species. Estimation and following of the mortality of the birds has been made difficult by a worsening of the weather conditions (severe drop in temperature, heavy rains followed by wind), so that in the second week of the observation a slight increase of mortality has appeared, but based on section of dead animals (72 from B group, 61 from C group, and 60 from A group) and parasitology examination of dead animals and group excrement from experimental groups there was no coccidiosis found.

Conclusion

Based on experiments performed using preparation A for use of the prevention and eradication of coccisiosis in pheasant game birds in intensive upbringing we can conclude the following:

- The preparation in the concentration of 0.25 gr/kg of food can be successfully used in prevention of this disease. The preparation should be used by adding it to the food within 7 days;
- The preparation in the concentration of 0.50 gr/kg of food can be successfully used during the beginning and clinically manifested form of coccidiosis. Therapy should be applied for 7 days;
- The preparation mixes easily with the food. The smell of the preparation is strong, specifically, of oregano, but in the food it cannot be tasted. Pheasants normally eat food with preparation mixed in it;
- In treated pheasants there were no side effects noticed as a consequence of the application of this preparation;
- The preparation can be applied both for preventive or therapeutical use, for the diseases of bacteridical or mycological ethiology of game birds. Based on shown coccidiostatic effect it may be applied for this use, like with application of other coccidiostatics (from third to eighth week of upbringing).

D) EXAMPLES CONCERNING THE SAFETY OF VETERINARY PHARMACEUTICAL MEDICAMENTS ACCORDING TO THE INVENTION

EXAMPLE 16

1. Introduction

In this example a study is carried out to get more information about the safety of pharmaceutical compositions comprising etheric oil of *Origanum vulgaris* (oregano oil) in broiler chicks. For this purpose two dietry levels were included in the trail: the normal recommended dose level of 250 ppm oregano oil and a dose level 10 times the recommended level (2,500 ppm of oregano oil). The latter dose level is generally used for testing the safety of products in order to apply for EU-registration referring to Council directive (COM C93 113) and Council decision (COM (93) 114). As a reference product, a commercially used antibiotic (virginiamycin; 20 ppm) was included in the trail. Birds were housed in battery cages for a period of 34 days. The criteria studied were weight gain, feed intake, feed conversion efficiency, water intake and general state of health.

2. Experimental Procedure

2.1. Experimental Groups

The following four experimental groups were involved in the trial:

Group	Diet
I	Basal diet (control diet without antibiotics)
II	Basal diet + 250 ppm oregano oil
III	Basal diet + 2,500 ppm oregano oil
IV	Basal diet + 20 ppm virginiamycin

Each experimental group consisted of 90 thicks, six replicate cages each with 15 female birds.

2.2 Animals

A normal broiler cross ("Ross") was used. At the time of arrival at the institute, 460 one day old female birds were divided at random among 24 cages. During the pre-test period of 5 days all birds were fed a standard diet. At an age of 5 days 360 birds were selected and divided at random among the experimental groups according to body weight. The allocation was done in such a way as to obtain within each group two cages with 15 birds of an average body weight of 114 g, two with an average body weight of 107 g, and two with an average body weight of 102 g. After allocation the chickens were fed the experimental diets for 29 days (age period 5–34 days).

2.3 Variations

The birds were vaccinated for Newcastle disease (according to the spray method) at one and fourteen days.

2.4 Housing

The birds were housed in battery cages, situated in an artificially heated, ventilated and lighted broiler house. The broiler house involved 72 cages. The floor space of each cage was 0.98 square meters with wire floors. Each cage was provided with an automatic water supplier and a feed trough. Per cage, 15 birds were housed. The broiler house was illuminated 24 hours a day. During the experimental period, the light intensity was gradually reduced. The temperature in the broiler unit was gradually reduced from 28° C. during the first week to 23° C. during the last days of the experiment. The humidity in the broiler unit was approximately 55% during the experimental period.

2.5 Diets

For the experiment one batch of feedstuffs were used. The composition of the basic diet is presented in Table B. No coccidiostat was added to the diets.

The experimental diets were prepared at a feed mixing plant. The basal diet of the experimental groups was mixed as one batch for all groups. The experimental diets were then prepared by splitting up this batch into four batches to which the required amount of feed additives were added, and mixed. Next the diets were pelleted (2.5 mm) without the addition of steam. The pelleting temperature, measured after the pellets left the press, was approximately 54° C. The basal diet was analysed for the content of crude protein, Ca and P.

The diets were fed ad libitum. Water was also available ad libitum via an automatic device.

TABLE B

Composition of the basal diet (in %)	
Ingredient	
Wheat	35.00
Corn	10.00

TABLE B-continued

Composition of the basal diet (in %)		
Soya oil	3.10	
Animal fat	3.00	
Tapioca	3.94	
Peas	10.00	
Soyabean meal (47.6% CP)	15.00	
Soyabean heattreated	5.00	
Sunflower meal	5.00	
Meat meal tankage (58% CP)	5.00	
Feathermeal (hydr. 82% CP)	1.50	
Vitamin-mineral mix*	1.00	
Limestone	0.88	
Monocalciumphosphate	0.92	
Salt	0.26	
L-lysine HCl	0.20	
Dl-methionine	0.20	
Crude protein	22.3	(21.9)
Dig. crude protein	18.3	
ME broilers (kcal/kg)	2900	
ME roosters (kcal/kg)	3140	
Crude fat	9.4	
Crude fibre	3.6	
Ash	5.8	
Calcium	0.86	(0.85)
Phosphorus	0.71	(0.73)
Available P	0.45	
Sodium	0.16	
Potassium	0.85	
Chloride	0.28	
Magnesium	0.16	
Linoleic acid	3.0	

Amino acids:	Total	AFD**
Lysine	1.28	1.09
Methionine	0.54	0.48
Meth. + Cyst.	0.94	0.79
Threonine	0.82	0.67
Tryptophan	0.24	0.20
Isoleucine	0.90	0.76
Leucine	1.65	1.40
Phenylalanine	1.03	0.88
Tyrosine	0.70	0.58
Valine	1.06	0.87
Arginine	1.48	1.28
Histidine	0.51	0.43

*Supplied per kg diet: riboflavin, 4 mg; niacinamide. 40 mg; d-pantothenic acid, 12 mg; choline-chloride, 500 mg; cobalamin. 15 μg; D1-α-tocopheryl acetate. 15 mg; menadione 5 mg; retinyl-acetate, 3.44 mg; cholecalciferol, 50 μg; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4.7N_2O$, 300 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$, 100 mg; $ZnSO_4.H_2O$, 150 mg; Na_2SeO_3, 0.15 mg; KI, 5 mg; $CoSO_4.7H_2O$,
1 mg: antioxidant (ethoxyquin), 100 mg; and 20 mg virginiamycin in phase 2.
**Apparent faecal digestible amino acids.
( ) Analysed contents

3. Criteria Studied

Individual body weight after an experimental period of 14 and 29 days.

Feed consumption for each replicate of 15 birds, at each time of weighing.

Feed conversion efficiency, calculated as kg feed consumed/kg weight gain, at each time of weighing. The data for feed consumption and conversion efficiency were corrected for the estimated amount of feed consumed by birds which died during the experimental period.

Water consumption and feed intake for each replicate of 15 birds, during one period of four days (24–28 days of age).

Mortality rate, and general state of health.

4. Statistical Analysys

The results for weight gain, feed conversion efficiency, feed intake and water consumption were analysed statistically. Results for weight gain, daily feed intake, and feed conversion efficiency are corrected for sex errors and 'outliers'. Differences among experimental groups were tested for significance by analysis of variance followed by the Least Significance Difference test (Snedecor and Cochran, 1980). The computer program SPSS/PC+ V5.0 (Norusis, 1992) was used to calculate the analysis of variance. All statements of significance are based on a probability of $P \leqq 0.05$.

5. Conclusion

The results for weight gain, daily feed intake, and feed conversion efficiency at 14 and 29 days experimental period are presented in Tables C and D. Supplementation of either 250 or 2,500 ppm of organo oil to the diet had hardly any effect on eight gain and feed conversion efficiency after 14 and 29 days experimental period. Virginiamycin when added to the diet tended to improve weight gain and feed conversion efficiency after 14 days experimental period, whereas hardly any effect on broiler performance was obtained after 29 days experimental period. The results for water intake and water/feed ratio are presented in Table E. Inclusion of either 250 and 2,500 ppm of oregano oil, or 20 ppm virginiamycin in the diet had hardly any effect of daily water intake and water/feed ratio.

Mortality rate was low, 1.4% (=5 animals), with no appreciable differences among the treatment groups. In addition, no abnormalities regarding the health status were observed during the trial. The low mortality rate obtained during the present trial indicates that the animals were in good health condition. This good health condition of the birds in the present study may explain the fact that hardly any effect of virginiamycin on broiler performance was observed, whereas normally an improvement in feed conversion efficiency is observed. Based on the results of the present study it can be concluded that oregano oil has no negative or detrimental effect on performance of healthy broiler chicks when supplemented 10 times the recommended level.

TABLE C

Results for body weight gain, daily feed intake, and feed conversion efficiency of broiler chicks after 14 days experimental period (5–19 days of age).

		Weight gain		Feed intake		Feed gain	
Group	Addition of	(g)	%	(g/d)	%	ratio	%
I	—	585	100	60.6	100	1.450	100
II	250 ppm Origanum oil	577	98.6	59.4	98.0	1.441	99.4
III	2,500 ppm Origanum oil	582	99.5	60.3	99.6	1.452	100.1
IV	20 ppm virginiamycin	592	101.1	60.5	99.8	1.431	98.7
LSD (P = 0.05)		14		1.0		0.021	

TABLE D

Results for body weight gain, daily feed intake, and feed conversion efficiency of broiler chicks after 29 days experimental period (5–34 days of age).

		Weight gain		Feed intake		Feed gain	
Group	Addition of	(g)	%	(g/d)	%	ratio	%
I	—	1590	100	93.7	100	1.710	100
II	250 ppm Origanum oil	1571	98.8	92.2	98.3	1.702	99.5
III	2,500 ppm Origanum oil	1593	100.2	94.0	100.3	1.711	100.1
IV	20 ppm virginiamycin	1606	100.3	93.4	99.6	1.697	993
LSD (P = 0.05)		38		1.9		0.024	

TABLE E

Results for water consumption and water feed ratio of broiler chicks during one period of four days (24–28 days of age).

		Daily water intake		Water/feed ratio	
Group	Addition of	(g/d)	%	ratio	%
I	—	291	100	2.06	100
II	250 ppm Origanum oil	295	101.3	2.07	100.7
III	2,500 ppm Origanum oil	295	100.3	2.09	101.4
IV	20 ppm virginiamycin	290	99.6	2.08	101.2
LSD (P = 0.05)		13.2		0.07	

What is claimed is:

1. A method of reducing the incidence of or treating a gastrointestinal infection in birds or mammals comprising orally administering to said birds or mammals an effective amount of a pharmaceutical composition, wherein the composition consists essentially of an active agent and a pharmaceutically acceptable carrier, wherein the active agent is an oil consisting essentially of a combination of thymol and carvacrol, said oil being extracted from *Origanum vulgaris* plants, wherein the oil is 1–15% by weight of the total weight of the pharmaceutical composition.

2. The method of claim 1, wherein the oil is 4–10% by weight of the total weight of the pharmaceutical composition.

3. The method of claim 1, wherein the pharmaceutical carrier is selected from the group consisting of lactose, honey, laurel, vaseline, paraffin, and calcium carbonate.

4. The method of claim 1, wherein the birds are poultry.

5. The method of claim 4, wherein the method is to treat a gastrointestinal infection, wherein said gastrointestinal infection is coccidiosis.

6. The method of claim 4, wherein the method is to reduce the incidence of a gastrointestinal infection, wherein said gastrointestinal infection is coccidiosis.

7. A pharmaceutical composition for oral administration in a veterinary application, consisting essentially of a pharmaceutically acceptable carrier, *Origanum vulgaris* oil 3–5% by weight of the total pharmaceutical composition, *Thymus vulgaris* oil 1–3% by weight of the total pharmaceutical composition, and *Mentha piperita* oil 0.5–1.5% by weight of the total pharmaceutical composition.

8. A method of treating coccidiosis in poultry, comprising the step of administering an effective amount of the composition of claim 7.

9. A method of reducing the incidence of coccidiosis in poultry comprising orally administering an effective amount of a pharmaceutical composition to said poultry, wherein the composition consists essentially of an active agent and a pharmaceutically acceptable carrier, wherein the active agent is an oil consisting essentially of a combination of thymol and carvacrol, said oil being extracted from *Origanum vulgaris* plants.

10. The method of claim 9, wherein the oil is 4–10% by weight of the total weight of the pharmaceutical composition.

* * * * *